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A Cytomorphological Study of Podospora curvicolla (Winter) Niessl

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A CYTOMORPHOLOGICAL STUDY OF PODOSPORA CURVICOLLA (WINTER) NIESSL

(TITLE)

BY

STEPHEN F. TOMASINO

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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A CYTOMORPHOLOGICAL STUDY OF *PODOSPORA CURVICOLLA* (WINTER) NIESSL

BY

STEPHEN F. TOMASINO

B.S. in Botany. Eastern Illinois University, 1980

ABSTRACT OF A THESIS

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ABSTRACT

A cytomorphological study was performed on Podospora curvicolla, a saprophytic Pyrenomycete. Major emphasis was placed on three important features: (I) ascocarp formation and development, (II) centrum structure and (III) ascus development and structure. A comparison with other studies of Podospora and with a genus generally considered closely related, Sordaria, was made to this present study of P. curvicolla.

Initial perithecial formation in P. curvicolla is indicated by a spherical mass of hyphae which may include filaments from many vegetative hyphae, but no apparent ascogonia or antheridia were observed. As the young perithecium matures in P. curvicolla, differentiation of the perithecial wall cells, the pseudoparenchymatous tissue and binucleate ascogenous cells takes place.

Developmental studies of other Podospora species indicate a few differences in ascocarp formation and perithecial structure in comparison to P. curvicolla. In both P. anserina, studied by Mainwaring and Wilson (1968), and P. arizonensis, researched by Mai (1976), coiled ascogonia and paraphyses were reported, features not observed in

Podospora curvicolla. Carr and Olive researched Sordaria fimicola in 1957 and they reported that the developemnt of the ascocarp of S. fimicola showed distinct similarities to that of P. anserina and P. arizonensis, this includes the formation of a coiled ascogonium and paraphyses. Thus, it is the first stage of ascocarp development in P. anserina, P. arizonensis and S. fimicola that differs significantly from P. curvicolla. Another major difference is the presence of paraphyses in P. anserina, P. arizonensis and S. fimicola, which are lacking in P. curvicolla. The later development of the young perithecium appears to be essentially the same in all three species of Podospora and in S. fimicola.

P. curvicolla follows closely the characteristics outlined for the Diaporthe type centrum. The distinctive ascocarp formation, presence of a pseudoparenchymatous centrum, formation of a perithecial wall and the eventual basal location of the ascogenous tissue provides adequate reasoning for assigning P. curvicolla to the Diaporthe centrum type. It should be noted that Mainwaring and Wilson (1968) treated P. arizonensis as having a Diaporthe centrum type while Mai (1976) considered P. anserina as an intermediate between the Diaporthe and Xylaria centrum types. As reported by Luttrell (1951), S. fimicola is also assigned to the Diaporthe centrum type.

Ascus development in P. curvicolla appears to closely follow that reported in other Podospora species studied. The asci develop from typical croziers and after growth occupy a large portion of the mature centrum.

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INTRODUCTION

The genus Podospora is a saprophytic Pyrenomycete which has received only limited morphological study. In the most recent monograph of the genus Podospora, Mirza and Cain (1969) recognize 64 species. This non-stromatic genus is mainly coprophilous with many species living on the dung of grazing animals. The perithecia are dark colored, occasionally tomentose, often with setae around the ostiole and are generally partially sunken into the substrate. The asci are unitunicate and are commonly clavate in shape, with some species exhibiting cylindrical asci. Cylindrical asci normally contain 4 to 8 ascospores while the clavate types have up to 512 ascospores. The ascospores are generally oval in shape and are dark colored. Primary, hyaline appendages are found at the base of the ascospores, with some species having secondary appendages attached at the apex. Ascospores of Podospora also have an apical germ pore present. Sordaria, generally considered a closely related genus, lacks appendages on the ascospores.

The genus Podospora was first established by Cesati in 1856 with the type species being Podospora fimicola

Cesati. This newly established genus had appendages present on the ascospores. However, Cesati and DeNotaris in 1863 described the genus Sordaria and used this generic name to represent those coprophilous Sphaeriales both with and without ascospore appendages. Ellis and Everhart (1892) treated Podospora and Sordaria as separate genera based on the presence or absence of ascospore appendages and placed both genera in the family Sordariaceae. Gwynne-Vaughan (1922) also adopted this same classification and placed the family Sordariaceae in the order Sphaeriales. This treatment was varied slightly by Clements and Shear (1931) by using the family name Sphaeriaceae. Cain (1934) combined the two genera under the name Sordaria, as did Gauman (1952) and Von Arx (1954). Gauman placed Sordaria in the Sordariaceae while Von Arx used the family Xylariaceae. Anders and Munk (1957) separated Podospora from Sordaria but used the name Pleurage Kuntze to represent Podospora. The invalid generic name Pleurage is still used occasionally in the mycological literature. Cain (1961) revised the genus Sordaria to include Podospora as a valid genus in the Soradariaceae. Wehmeyer (1975) also separated the two genera and placed Podospora in the Sordariaceae and Xylariales. Bessey (1935), an early textbook author, used Pleurage to represent Podospora and placed the genus in the Fimetariaceae and Sphaeriales. Textbook authors Alexopoulos (1979) and Webster (1980) both recognize Podospora and Sordaria as separate genera and,

following tradition, use the family Sordariaceae. Webster places the family in the Sphaeriales while Alexopoulos uses Xylariales.

This study pertains to Podospora curvicolla (Winter) Niessl in which developmental morphology and cytology are described. In nature, Podospora curvicolla, like most species in the genus, grows on the dung of animals. The perithecia are pear-shaped and sometimes have an elongated neck while scattered setae are found around the ostiole at maturity. The asci are clavate in shape and contain up to 256 ascospores. Asci generally range from 250-300 x 85-100 microns in size. The spores are ellipsoid in shape and are dark brown in color and attain a size of 14-16 x 9-11 microns. Each spore has a slender, short, primary appendage attached at its base, with a smaller, secondary appendage at its apex.

LITERATURE REVIEW

A large amount of taxonomic, genetic, cytological, and morphological research has been performed on Sordaria, a genus usually regarded as closely related to Podospora. In contrast, only a limited number of cytomorphological studies have been performed on Podospora despite some species being somewhat common. The first detailed study of the genus was performed by Beckett and Wilson (1968), using P. anserina (Ces.) Rehm. to study the ascus cytology. In their research, typical croziers were observed. They reported the fusion of nuclei in the penultimate cell after the penultimate had enlarged. Also noted was the fusion of the ultimate cell with the antepenultimate cell and the fusion of their nuclei. A haploid chromosome number of seven was observed during metaphase I. The study was carried through two meiotic divisions and one mitotic division of nuclei in the developing ascus to the formation of ascospores.

Mainwaring and Wilson (1968) performed a similar study on P. arizonensis (Griff.) Cain. They noted that this species has asci containing four large black and four small hyaline ascospores. The study reported that the small spores did not germinate while the large spores showed a low percent

of germination after a period of dormancy. It was noted that the ascus arises from a binucleate penultimate cell of the crozier, but the fusion of the two nuclei does not occur. Instead of meiosis, the nuclei undergo two mitotic divisions to give eight haploid ascospores. The perithecium followed the Diaporthe type development. An ascogonial coil and a pseudotrichogyne were reported.

Podospora anserina was used by Mai (1976), who studied its overall morphology. He noted that a coiled ascogonium initial formed from vegetative mycelium. After this coiled initial had formed, hyphae grew up and around the initial. Eventually, the initial was enveloped by one or two hyphal layers. These hyphae originated from the filament from which the coil had arisen and also from adjacent filaments. The layered hyphae later became the perithecial wall. Mai reported a layer of pseudoparenchyma cells which formed inside the developing perithecium, with many paraphyses filling the young centrum. As the perithecium matured, he observed a compact mass of ascogenous cells at the base of the perithecium. These ascogenous cells were somewhat central at first but expanded laterally as the perithecium grew. He noted that the development of the perithecium of P. anserina was very similar to that of P. arizonensis reported by Mainwaring and Wilson (1968).

MATERIALS AND METHODS

The culture of Podospora curvicolla was obtained from the Eastern Illinois University fungus culture collection. The initial collection and identification was made by Dr. Alan Parker of the University of Wisconson-Waukesha.

The fungus was grown in an oatmeal and agar combination. The media was prepared by autoclaving petri dishes which contained 10-15 oatmeal flakes. A 1.3-1.5% agar mixture was prepared, autoclaved and poured into petri dishes over the sterilized oatmeal flakes. It was important that the flakes were kept scattered evenly throughout the plate to allow for better mycelium growth. The agar depth was not a critical factor but the dishes were filled at least half full.

The agar plates were inoculated by inserting a small sample of mycelium or spores into various areas on the plate, usually four locations were sufficient. The cultures were grown at room temperature. It was determined that at temperatures above 85°F the development of the perithecia was slow or did not occur at all. The optimal growing temperature was between 70-75°F. Mycelium growth was observed three days

after inoculation. The dark, olive-green colored mycelium grew rapidly in a circular fashion about the point of inoculation. Young ascogonial initials could be found microscopically on hyphae after seven to nine days of growth. Perithecia appeared thirteen to fifteen days after inoculation. It was very common for perithecia to only form in the direct vicinity of an oatmeal flake. Healthy cultures continued to grow in unopened agar plates for up to two months.

The young ascocarps were studied by removing a small section of agar from an area on the plate where young perithecia could be seen. A low powered dissecting scope aided in the observation of areas of young perithecia. A small portion of agar was then placed in a drop of water on a slide and a cover slip was applied. Depending on the stage of development, the young ascocarps were viewed under high power (500X) and oil immersion (1250X).

Ascogenous hyphae were demonstrated by using two smear techniques, the propiono-carmine and the Giesma stain techniques. Young perithecia, that is those without mature ascospores, were obtained by cutting small blocks of agar out of the plates containing perithecia. These young perithecia appeared lighter in color and almost transparent as compared to the dark, thick walled, mature perithecia. The agar blocks containing the perithecia were placed into a killing and fixing solution. The solution used was the

formalin-aceto-alcohol mixture (Johansen, 1940). The perithecia were left in the fixative for 24-36 hours. During this time, the container was placed in a refrigerator.

In using the propiono-carmine technique, the blocks were placed in the propiono-carmine stain for three days after the fixation had been completed. Again, the material was placed in a refrigerator during the time period. After three days, a few perithecia were carefully dissected out of the agar blocks and were placed in a drop of propiono-carmine stain. The centrum portion of the perithecia was dissected out and the remaining parts of the perithecia were removed from the slide. A dissecting scope and beading needles attached to holders aided in the dissection. A cover slip was placed over the dissected centrum and light pressure was applied to enhance the separation of ascogenous hyphae and other cell types. Gentle heating of the slide over a flame and occasionally adding more stain to the slide were techniques used to intensify and set the stain. Then, more intense pressure was applied to the cover slip in an effort to further spread the cells. The cover slip was sealed by using nail polish or a sealent containing one part 45% glacial acetic acid, one part Karo syrup, and one part aqueous suspension of pectin. This procedure proved to be somewhat unsuccessful in demonstrating the nuclei of the ascogenous hyphae. However, general ascus structure could easily be determined. The major problem apparent with

the propiono-carmaine technique was the inability to separate ascogenous cells from larger cell types present.

The Giesma stain procedure proved to be much more valuable and easier to perform. The perithecia were again fixed for 24-36 hours under refrigeration and the centrum region was dissected out into a drop of water. Two drops of undiluted Giesma stain were placed on the dissected centrum and one drop of distilled water was also added. The mixture was left undisturbed for 1 to 2 minutes. The slide was flooded with water to remove excess stain and then a cover slip was placed on it. Gentle pressure was then applied on the cover slip to spread the cells. Often, shifting of the cover slip in a circular fashion was used to aid in the spreading of the cell mass. A variation of this technique was also performed. After the centrum had been dissected from the perithecium, it was placed into a drop of ribonuclease solution. The solution was prepared by adding .04 grams of the enzyme to 1 ml of distilled water. Preparation of this solution was performed immediately before use. The centrum material was left in the solution for 2 minutes and then it was drained off the slide with a paper towel. Two drops of Giesma stain and then two drops of distilled water were placed on the centrum material. A cover slip was placed on and gentle pressure was applied. This variation using ribonuclease was helpful in loosening the dense cellular mass which surrounded the ascogenous hyphae.

Sealed slides remained in good condition for one week.

The development of the perithecium and centrum were studied by sections cut with a rotary microtome. Small blocks of agar (1cm x .5cm) containing perithecia of the desired age were fixed and killed in Randolf's modification of Navashin fluid (Johansen, 1940). The perithecia were aspirated for 1 hour and left in the fluid for 18-24 hours. The blocks were then rinsed in tap water and dehydrated in an alcohol series before staining. The tertiary butyl alcohol method was used in the dehydration process (Johansen, 1940). The method used was slightly different than that of Johansen's. Grades were assigned to represent various alcohol concentrations with grade 1 having the lowest alcohol percentage and grades 10, 11, and 12 having the highest (Table 1). Grade 12 contains alcohol and paraffin oil which allows for infiltration of paraffin into the agar blocks. Some of the grade 12 solution with the agar blocks were placed into vials containing melted paraffin. The vials were then placed into an oven at 60°C. The paraffin was then replaced after the agar blocks had settled to the bottom of the vials. The vials were left in the oven overnight and again another change of paraffin was performed. The agar blocks were then placed in small ceramic dishes filled with hot paraffin. After all the paraffin had totally solidified, paraffin blocks containing perithecia were cut out and removed. These paraffin blocks were mounted on wooden blocks and

sectioned with the microtome at thickness of 12-15 microns. The ribbon strips were attached to slides with Haupt's adhesive, and the slides were allowed to dry on a warming tray for 6-12 hours. The paraffin was removed by placing the slides in two changes of xylene for 5 minutes each. Hydration of the material was accomplished by placing the slides in alcohol solutions according to the following schedule: 5 minutes each in 95, 80, 70, 50, 30, and 10% ethyl alcohol and finally 2 minutes each in two changes of distilled water. This completed the slide preparation before the staining process.

Two modified staining methods were performed, the Gram stain and Heidenhain's hematoxylin stain. For the Gram stain, the slides were placed in Gram stain (Bartholomew and Schneider, 1973) for 30 minutes. They were then rinsed in tap water and placed into a solution of iodine-potassium-iodide for 2 minutes (Johansen, 1940). Following a rinse in tap water, the slides were passed through 95% ethyl alcohol for destaining purposes. The purple color of the stain was not apparent after destaining. The slides were placed into clove oil for 10 seconds and finally in two changes of xylene which served as a clearing agent. Permount was used as the mounting medium for the cover slips.

Heidenhain's iron-alum hematoxylin stain was another method utilized. The slides were placed in 4% iron alum (ferric ammoniun sulfate) for 4 hours. After this time,

the slides were washed thoroughly in running tap water for 5 minutes and then rinsed in two changes of distilled water. The slides were then placed in a .5% hematoxylin stain for 12 hours (Johansen, 1940). Following the stain, the slides were washed off with two changes of distilled water and then destained. Destaining was carried out in 2% iron alum for 2-3 minutes. The time factor for destaining was critical to the overall success of the stain, and the slides were observed microscopically at intervals to determine when to remove the slides from the destaining solution. A thorough washing for 5 minutes in running tap water followed the destaining process. The slides were then dehydrated in the following alcohol series: 5 minutes each in 30, 50, 70, and 80% ethyl alcohol, followed by two changes in 100% ethyl alcohol. Two changes of xylene were used as a clearing agent and the cover slips were mounted on with Permount.

Both staining techniques worked well. The Gram stain provided excellent staining of ascogenous hyphae and associated nuclei. The hematoxylin proved valuable in staining the perithecial wall, periphyses and asci.

Homothallism was determined by using a sterile dilution technique. Mature asci were crushed and placed in a small container of sterile water (10 mls). The 10 mls were transfered into a test tube containing 90 mls of sterile water. The solution was mixed vigorously to separate the spores. Oatmeal agar plates were inoculated from this dilution,

with one or two drops of water from the test tube placed on each plate. The water was distributed about the plate using a sterile bent glass rod. After small colonies of the fungus were observed, they were transfered to separate plates for growth and development.

OBSERVATIONS

In this study, particular attention was given to three aspects of the cytomorphology of Podospora curvicolla: (I) ascocarp development, (II) centrum structure and (III) development and structure of the ascus. These three features, along with spore and setae discriptions, are important in the classification and taxonomy of the species in the genus.

I. The Ascocarp Development

Ascocarps were initiated seven to nine days after inoculation. A distinct pattern of hyphal development indicated the initial formation of the young perithecium. First, special hyphal branches showed a distinct twisted and curled form as they grew from the vegetative filaments (Figs. 1, 2), with a considerable number of these branches originating a short distance from each other. In addition, branches from adjacent hyphae also became incorporated into the developing ascocarp (Fig. 3). All of these branches grew rapidly amongst themselves. Structures resembling ascogonia or antheridia were not observed.

In approximately twelve days after inoculation, the young perithecium appears as a spherical mass of tightly woven hyphae (Figs, 4, 5, 6), with perithecia often developing close to each other and on the same filament. In sectioned material, fertile ascogenous cells or "Woronin hyphae" were detected in the young perithecia. These cells stained more intensely than the surrounding cell types and were often observed to be in a binucleate condition (Figs. 4, 7). In cultures twenty to twenty four days old, the perithecia were fully developed with mature ascospores (Figs. 8, 9). The mature perithecium is black in color with the neck region protruding above the substrate. Towards the end of maturity, setae form on the neck region of the perithecium, with these setae numbering few to numerous and ranging from 1 to 4 cells in width. Fully developed perithecia varied in size, ranging from 300-650 x 325-400 microns.

II. The Centrum Structure

Sectioned perithecia at various ages showed the development of the centrum structure. Three distinct zones or layers were observed in the perithecia of cultures fourteen to seventeen days old (Figs. 10, 11, 12, 13). A pseudoparenchymatous layer, 3 to 4 cells thick, compose the outside perithecial wall, with the cells in this region darkly

pigmented and relatively small. The cells in this region were 1.5-2.0 microns in diameter. Pseudoparenchymatous cells are characterized by being closely compacted and irregularly oval in shape as opposed to prosenchymatous cells which are elongated cells and appear loosely woven or fiber-like. Along the neck region, setae arise from this layer of cells. Located beneath this first zone is a second layer. 2 to 3 cells thick, consisting of prosenchymatous cells giving an interwoven effect and staining much lighter than the cells of the outer layer. Cells in the second zone measured 1.5-2.5 microns in length and .5-1 micron in width. Periphyses originated from cells of this second layer along the inside apical area of the perithecium. A third distinct zone is the central portion of the perithecium, the centrum. This area is occupied by large, thin-walled pseudoparenchymatous cells which stain very lightly or not at all.

In young perithecia. the ascogenous cells develop in a central position, with pseudoparenchymatous cells surrouding them (Figs. 10, 11). The ascogenous cells tended to stained more intensely than the pseudoparenchymatous cells. As the perithecium matures, the ascogenous hyphae and asci become more basal in position while the pseudoparenchymatous cells occupy the upper portion of the centrum (Figs. 12, 13). As the asci develop, they push their way up through these large pseudoparenchymatous cells and occupy a large portion of the centrum while most of the pseudoparenchy-

matous cells appear to become crushed during elongation and enlargement of the asci. Sections of very mature perithecia still showed some of these large pseudoparenchymatous cells around the mature asci.

III. The Ascus

The stages of ascus development were observed using smear techniques with young perithecia from cultures seventeen to twenty days old. Development of the ascus is in the normal fashion by means of a three-celled crozier (Figs. 16, 17, 18, 19, 20). This hooked structure consists of a uninucleate ultimate cell, a binucleate penultimate cell and a uninucleate antepenultimate cell. The croziers originate from binucleate cells which develop centrally in the young perithecium (Figs. 14, 15, 21). Large numbers of the binucleate cells and crozier-like structures were observed in smears of young perithecia. The penultimate cell appears to develop into an ascus while the ultimate and antepenultimate cells fuse, resulting in another ascus or a new crozier. The resulting diploid nucleus or fusion nucleus undergoes meiosis and is then followed by mitotic divisions resulting in up to 256 haploid ascospores. The ascospores are dark brown, oval and have a gelatinous appendage attached at the base. The ascospores were 12-14 x 8-10 microns in size, with the appendage being 5-7 microns in length (Fig.

22). The mature asci are numerous, clavate in shape and occupy a large portion of the mature perithecium. Mature asci attained a size of 200-275 x 60-110 microns. Forcible spore discharge was evident by the accumulation of ascospores on the lid of the petri dish.

DISCUSSION

Detailed cytological and morphological studies of Podospora have been few in number. Presented here will be a comparison of studies previously carried out on other species of Podospora to this investigation of P. curvicolla. In addition, a comparison will be made with the well known genus Sordaria, a genus generally considered to be closely related to Podospora. Particular emphasis will be given to three important cytomorphological features: (I) ascocarp formation and development, (II) centrum structure, and (III) ascus development.

I. Ascocarp Formation and Development

Initial ascocarp development in P. curvicolla varies somewhat from the development reported for P. anserina and P. arizonensis. In P. arizonensis, Mainwaring and Wilson (1968) observed a multicellular ascogonial coil which arises from an intercalary cell of a vegetative hypha. They also described a nonfunctional pseudotrichogyne associated with most coiled ascogonia. This contrasts with the condition

in Podospora curvicolla where no coiled asogonia were observed. It is this first stage of ascocarp development that differs significantly from that of P. curvicolla, since later development of the young perithecium of P. arizonensis follows a pattern similar to that observed in P. curvicolla. This includes the growth of the vegetative hyphae to enclose the fertile cells, the formation of pseudoparenchymatous tissue and the orientation of the ascogenous cells in the young perithecium. However, paraphyses were reported in P. arizonensis, in contrast to P. curvicolla, where they could not be demonstrated. Ascocarp formation in P. anserina, as described by Mai (1976), follows closely to that of P. arizonensis. Mai reported a coiled ascogonium in P. anserina which arose from an intercalary cell of a vegetative hypha, and he noted the presence of a pseudotrichogyne. Subsequent development is essentially the same in both species.

Sordaria, being morphologically similar to Podospora but lacking spore appendages, can be compared to Podospora in regard to ascocarp development. Sordaria fimicola, an often studied species, was researched by Carr and Olive in 1957. Development of the ascocarp showed distinct similarities to that of P. anserina and P. arizonensis. Coiled ascogonia were observed originating from vegetative filaments with no discernible antheridia. They reported that the coil soon became surrounded and enveloped by hyphae. As development proceeded in S. fimicola, it was noticed in the young perithecium that deeper staining binucleate cells were

embedded in the mass of developing hyphae, a condition also observed in this present study of Podospora curvicolla. P. curvicolla appears to more closely follow the perithecial development found in species of Hypoxylon. The genus Hypoxylon is a stromatic Pyrenomycete and according to most authorities is usually placed in the Xylariaceae of the order Xylariales. Lupo (1922) studied the ascocarp development in H. coccineum and reported the formation of a circular knot of hyphae as the initial ascocarp. Within this mass of hyphae, "Woronin hyphae" differentiate and these cells develop into ascogonia. As previously mentioned, "Woronin hyphae" are dark staining, binucleate cells found in the central mass of hyphae of the young perithecia. This situation is very similar to results found in this study of P. curvicolla. Miller (1928) studied H. howeianum and found the ascocarp development to be essentially the same as in H. coccineum. Despite Hypoxylon being stromatic, the ascocarp development of P. curvicolla and species of Hypoxylon are obviously quite similar.

Due to the small number of developmental studies performed on Podospora species, it is difficult to formulate a valid statement comparing P. curvicolla to the genus as a whole. However, ascocarp development in P. curvicolla seems significantly different from that of the other two previously studied species of Podospora.

II. Centrum Structure

The centrum structure of Podospora curvicolla shows considerable similarity to the other Podospora species studied. As reported by Mainwaring and Wilson (1968), P. arizonensis exhibited pseudoparenchymatous tissue in the centrum which differentiates into a dark outer layer of cells and a lighter inner layer of cells to compose the perithecial wall. Thus, the perithecium is three-layered as in P. curvicolla. They also noted that the fertile tissue, once central in position, is pushed down towards the base of the perithecium as the thin-walled pseudoparenchymatous cells increase in size. Paraphyses were reported at the base while a cavity forms toward the apex of the centrum due to the separation of pseudoparenchymatous cells, with both of these conditions being absent in P. curvicolla. As in P. curvicolla, periphyses were also observed in the ostiole region of P. arizonensis.

Mai (1976), in his study of the morphology of P. anserina, found a similar centrum structure to P. arizonensis. He reports a parenchymatous centrum which differentiates into wall cells and broad paraphyses. The centrally located ascogenous cells in the young perithecium are forced downward during maturity, but no cavity was observed in the upper portion of the centrum. Mai states that the presence of the broad paraphyses which occupy the centrum with parenchymatous cells do not allow a cavity to form. Periphyses

were observed in Podospora anserina lining the ostiole region.

The various studies of Sordaria report much the same centrum condition found in P. anserina and P. arizonensis. Piehl (1928) describes the inner tissues of the young perithecia of S. fimicola as remaining thin-walled and the outer region to be thicker and darker staining. Piehl also reports the presence of hyphae which resemble paraphyses amongst the young asci, but these hyphae soon disintegrate as the asci mature. With reference to a study by Ritchie (1937), Luttrell (1951) states the centrum of S. fimicola as having pseudoparenchyma with a periphysate ostiole and no mention of paraphyses. However, Page (1939) mentions the formation of paraphyses in the centrum of S. fimicola. Uecker's (1976) study of S. humana reports the centrum to be much like that of S. fimicola. Uecker observed a central region of plectenchyma (pseudoparenchyma) and true paraphyses which disintegrate towards perithecial maturity.

It should be pointed out that the previous studies of Podospora species, as well as those of Sordaria, include only those with cylindrical asci which contain four to eight ascospores. Possibly, species such as P. curvicolla with clavate asci and large numbers of ascospores represent a distinct centrum condition with no apparent paraphyses or centrum cavity. Additional studies of species with both types of asci need to be carried out before valid conclusions can be made. In summary, the centrum structure of P.

curvicolla appears to develop very much like that of Podospora anserina and P. arizonensis despite the fact no paraphyses or centrum cavity were observed in P. curvicolla.

Luttrell's publication of 1951, which deals with Pyrenomycete taxonomy based on centrum morphology will be cited to further emphasize the importance of centrum structure to classification. Luttrell lists eight centrum types in the Pyrenomycetes, each based on the development of the ascocarp and on the composition and arrangement of cells in the centrum. One of these types is the Diaporthe type, the centrum type that P. curvicolla appears to follow. Genera exhibiting the Diaporthe condition have certain centrum characteristics in common. First, the young ascogonium is enveloped by hyphae from the ascogonial stalk or other vegetative filaments to form a spherical mass of hyphae, and this considered the perithecial initial. Second, the development of the initial includes the formation of a perithecial wall and a central region consisting of pseudoparenchymatous cells. The separation and disintegration of these pseudoparenchymatous cells results in a perithecial cavity, with basal ascogenous hyphae producing asci which grow up into this cavity. Variation in this centrum type include differences in initial ascocarp formation, fate of the pseudoparenchymatous cells, the presence or absence of paraphyses and the initial position of ascogenous cells.

P. curvicolla follows closely the characteristics outlined for the Diaporthe type centrum. However, P. curvicolla

exhibits a variation in initial ascocarp formation. The initial ascocarp involves the formation of a spherical mass of hyphae with no apparent ascogonium being formed. The filaments of this young perithecium differentiate into a perithecial wall, a central region of pseudoparenchyma and a number of binucleate ascogenous cells. The distinctive ascocarp formation, presence of a pseudoparenchymatous centrum, formation of a perithecial wall and the eventual basal location of ascogenous tissue provides adequate reasoning for assigning Podospora curvicolla to the Diaporthe centrum type. This centrum placement is repeated by Mainwaring and Wilson (1968) for P. arizonensis while Mai (1975) places P. anserina as an intermediate between the Diaporthe and Xylaria centrum types. Luttrell assigns Sordaria fimicola to the Diaporthe type based on morphological studies by Ritchie (1937), Dangeard (1907) and Page (1939).

The large amount of variation found in the centrum structure of many Pyrenomycetes has led to some confusion regarding taxonomic placement of many genera, and this is certainly the case in Podospora. Bessey (1950) placed Podospora in the Sphaeriales and Fimetariaceae based on the dark colored perithecial wall, the presence of pseudoparenchyma, the basal location of ascogenous tissue, the sunken location of the perithecia in the substrate and the lack of a stroma associated with the perithecium. Munk (1957), who uses the order Sphaeriales and the family Sordariaceae to accomodate Podospora, emphasizes that a great amount of

variation occurs in the centrum structure of this family. He reports the Diaporthe type in Sordaria, the Nectria type in Lasiosphaeria and the tendency for the the Xylaria type to be found in various other genera. Wehmeyer (1975) uses the order Xylariales to include both stromatic and non-stromatic forms of Pyrenomycetes and suggests that a number of non-stromatic genera are in fact closely related to stromatic forms. He separates the genera without a stroma or those having a poorly developed stroma into three families, and one of these is the Sordariaceae, in which Podospora is placed. Alexopoulos (1979) also uses the order Xylariales to contain both stromatic and non-stromatic genera with the Xylaria type centrum, and in this order he placed the family Sordariaceae to which he assigned Podospora and Sordaria. Alexopoulos follows Mai's (1976) interpretation of P. anserina as having an intermediate centrum type between the Diaporthe and Xylaria types, and thus he classifies the genus Podospora along with genera having a distinct Xylaria type centrum.

It is the opinion of this investigator that the stromatic and non-stromatic forms should be placed in separate families but not separate orders. Until more developmental studies on species of Podospora are undertaken, the genus Podospora is perphaps best placed in the Sordariaceae of the order Sphaeriales.

III. Ascus Development

Ascus development in Podospora curvicolla appears to closely follow that reported in other Podospora species studied. Beckett and Wilson (1968) observed typical three celled crozier formation in P. anserina, with the two penultimate nuclei fusing and the resulting cell forming into an ascus. They note also the fusion of the ultimate cell with the antepenultimate cell to form an ascus or a new crozier. Meiosis and the resulting mitotic division were observed during spore formation. Mainwaring and Wilson (1968) studied the ascus development in P. arizonensis and noted this species has the ascus arising in the normal fashion by means of a hooked crozier. However, they point out that the fusion of the two nuclei in the penultimate cell does not occur, rather two mitotic divisions result in eight haploid ascospores. Mai (1976) reported typical ascus development in P. anserina.

For the sake of comparison, Carr and Olive (1958) found crozier formation and ascus development in Sordaria fimicola to be typical for Ascomycetes. They reported meiosis and two mitotic divisions occurring amongst spore nuclei resulting in eight haploid ascospores.

With the formation of hooked croziers and subsequent fusion of crozier cells and nuclei to form asci, it appears that P. curvicolla follows a pattern of ascus development similar to many Ascomycetes.

SUMMARY

Initial perithecial formation in Podospora curvicolla is indicated by a spherical mass of hyphae which may include filaments from many vegetative hyphae. As the young perithecium matures, differentiation of the perithecial wall cells, the pseudoparenchymatous tissue and the binucleate ascogenous cells takes place. During development, the ascogenous tissue becomes more basal in position as the pseudoparenchymatous tissue occupies the upper portion of the centrum. Asci develop from typical croziers formed from the ascogenous hyphae and force their way up through the pseudoparenchyma. As the asci develop, the pseudoparenchyma is crushed and the mature asci occupy a large part of the centrum. Periphyses form in the ostiole region of the perithecium while basal paraphyses were not observed.

Developmental studies of other Podospora species indicate a few differences in ascocarp formation and perithecial structure in comparison to P. curvicolla. P. anserina, studied by Mainwaring and Wilson (1968), and P. arizonensis, researched by Mai (1975), both develop coiled ascogonia and paraphyses, features not observed in P. curvicolla. Despite the lack of paraphyses in P. curvicolla, the centrum

structure of P. curvicolla appears similar to the structure in P. anserina and P. arizonensis. The Diaporthe type centrum was assigned to P. curvicolla on the basis of the presence of a distinct perithecial wall, pseudoparenchymatous cells and the basal location of the ascogenous tissue.

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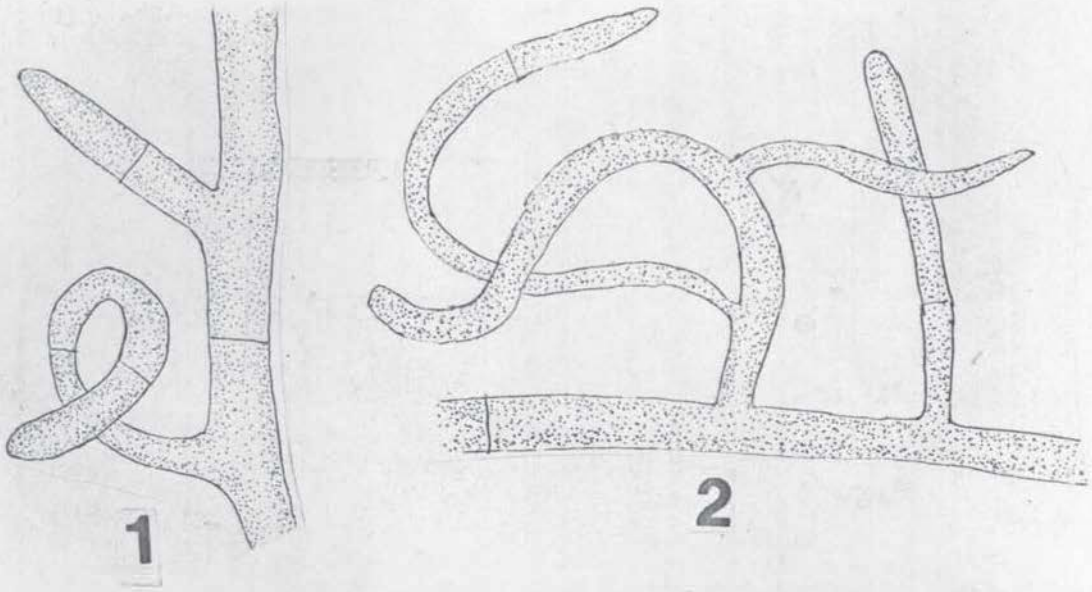
APPENDIX

EXPLANATION OF PLATES

Figures 1-3 — Initial ascocarp filaments. These figures show the twisting hyphae. X1250.

Figure 4 — Formation of binucleate ascogenous cells within the dense hyphae of the young perithecium. X1250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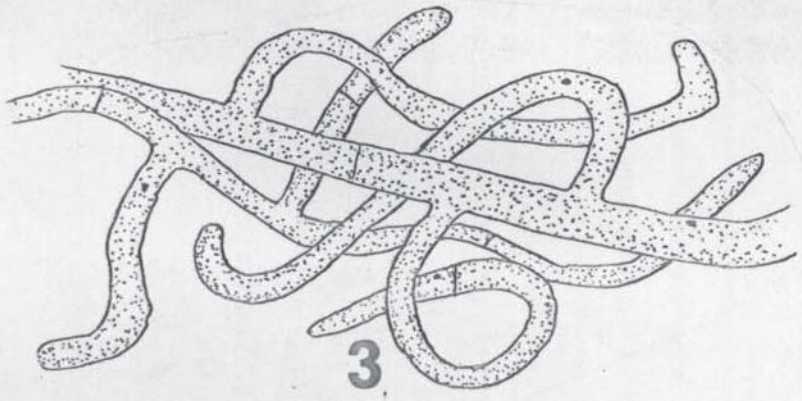
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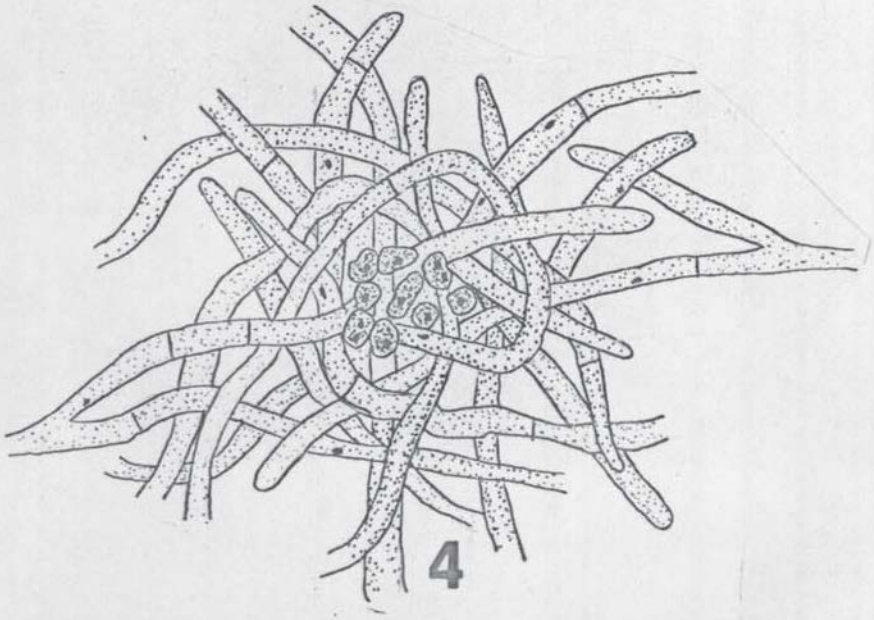
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EXPLANATION OF PLATES

Figures 5-6 — Developing perithecia showing compact mass of hyphae. X100.

Figure 7 — Section of a young perithecium showing binucleate "Woronin hyphae". The arrows indicate the position of nuclei. X100.

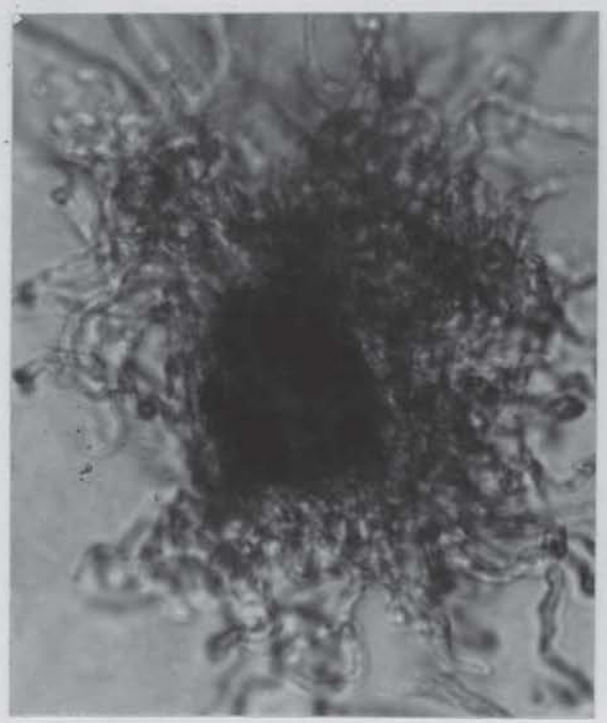


Fig. 5

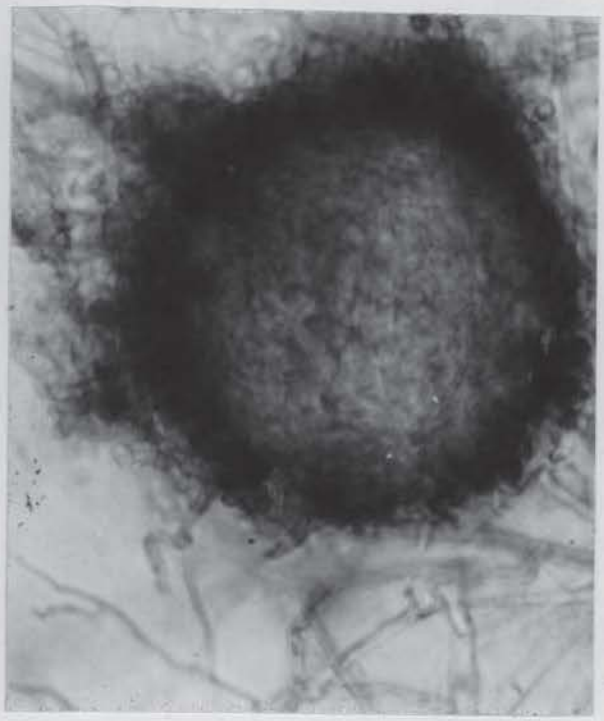


Fig. 6

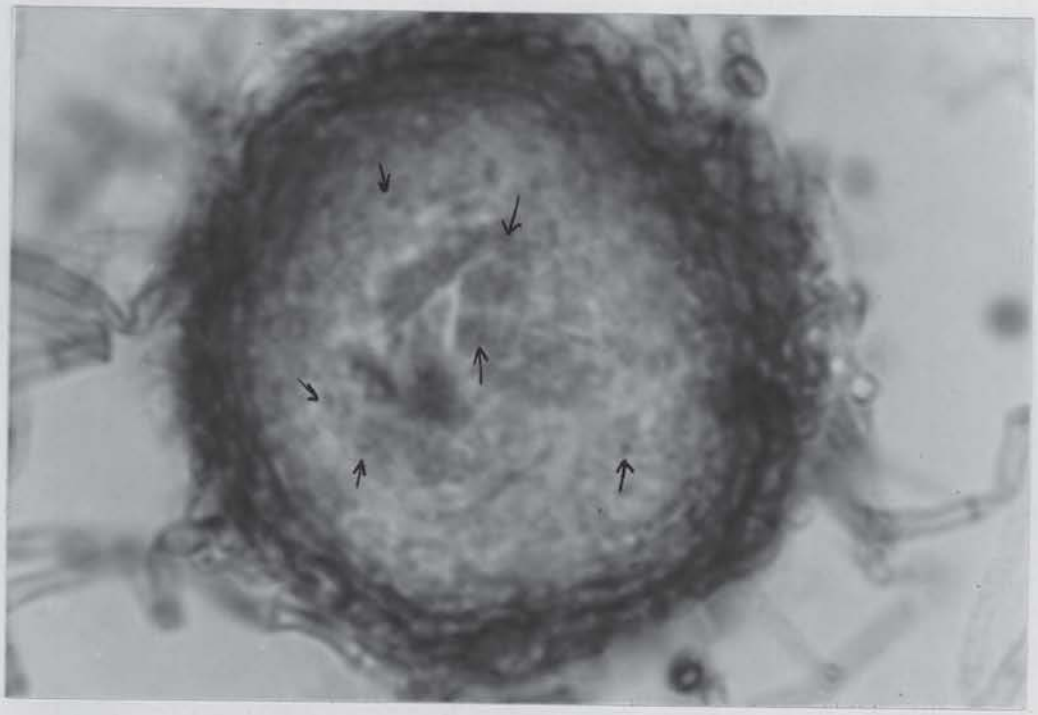


Fig. 7

EXPLANTAION OF PLATES

Figure 8 — Inmature perithecium approximately twenty days after inoculation. X40

Figure 9 — Fully mature perithecium approximately twenty four days after inoculation. X40

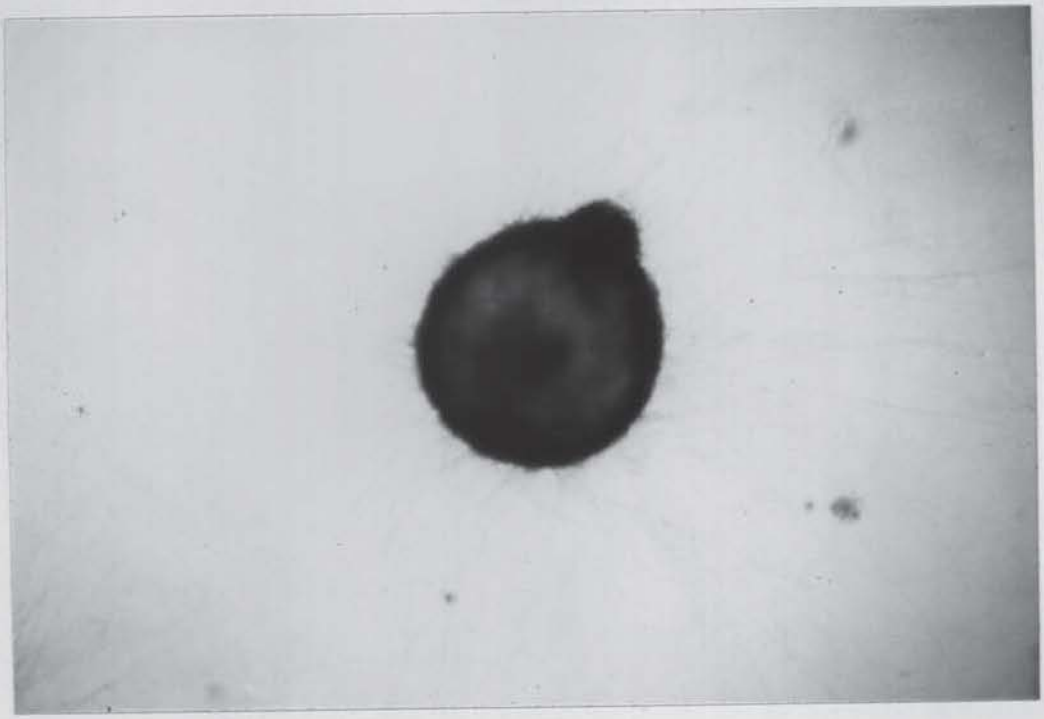
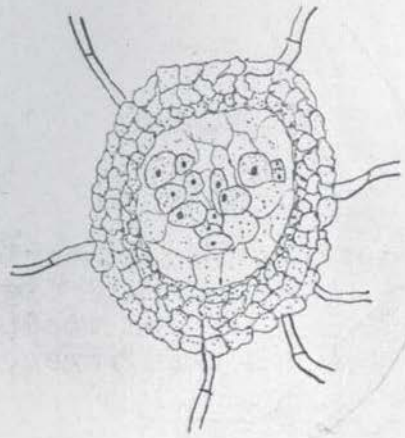


Fig. 8

Fig. 9

EXPLANATION OF PLATES

Figures 10-12 — Sectioned perithecia at various ages in development. All figures show the three-layered condition of the centrum and different cell types. X125.



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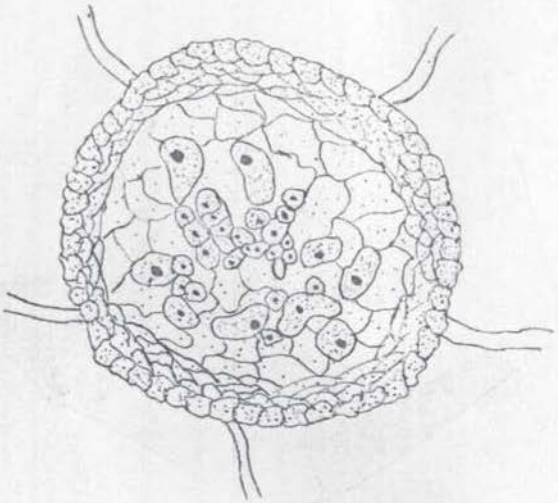

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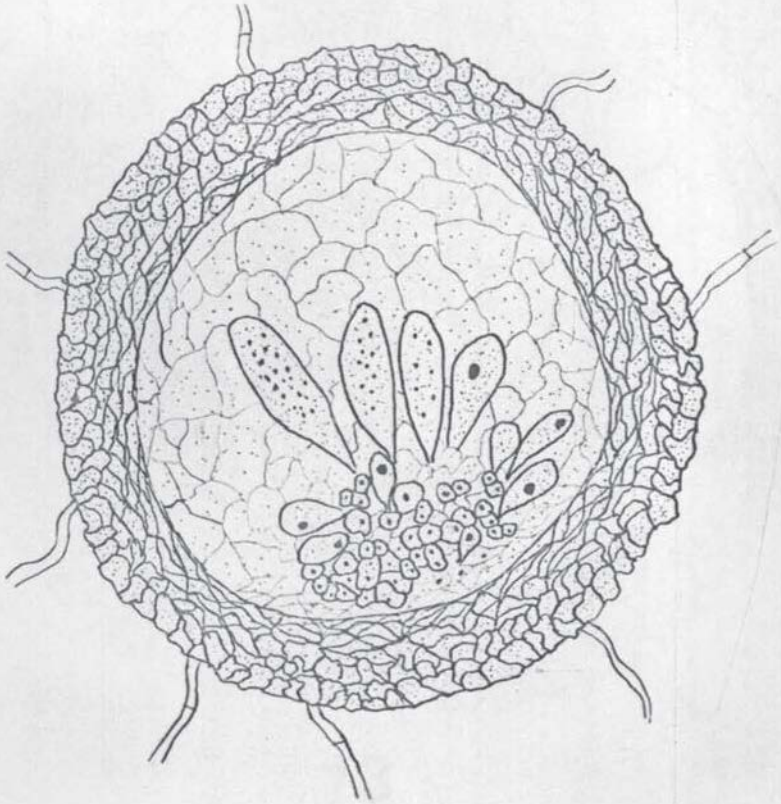
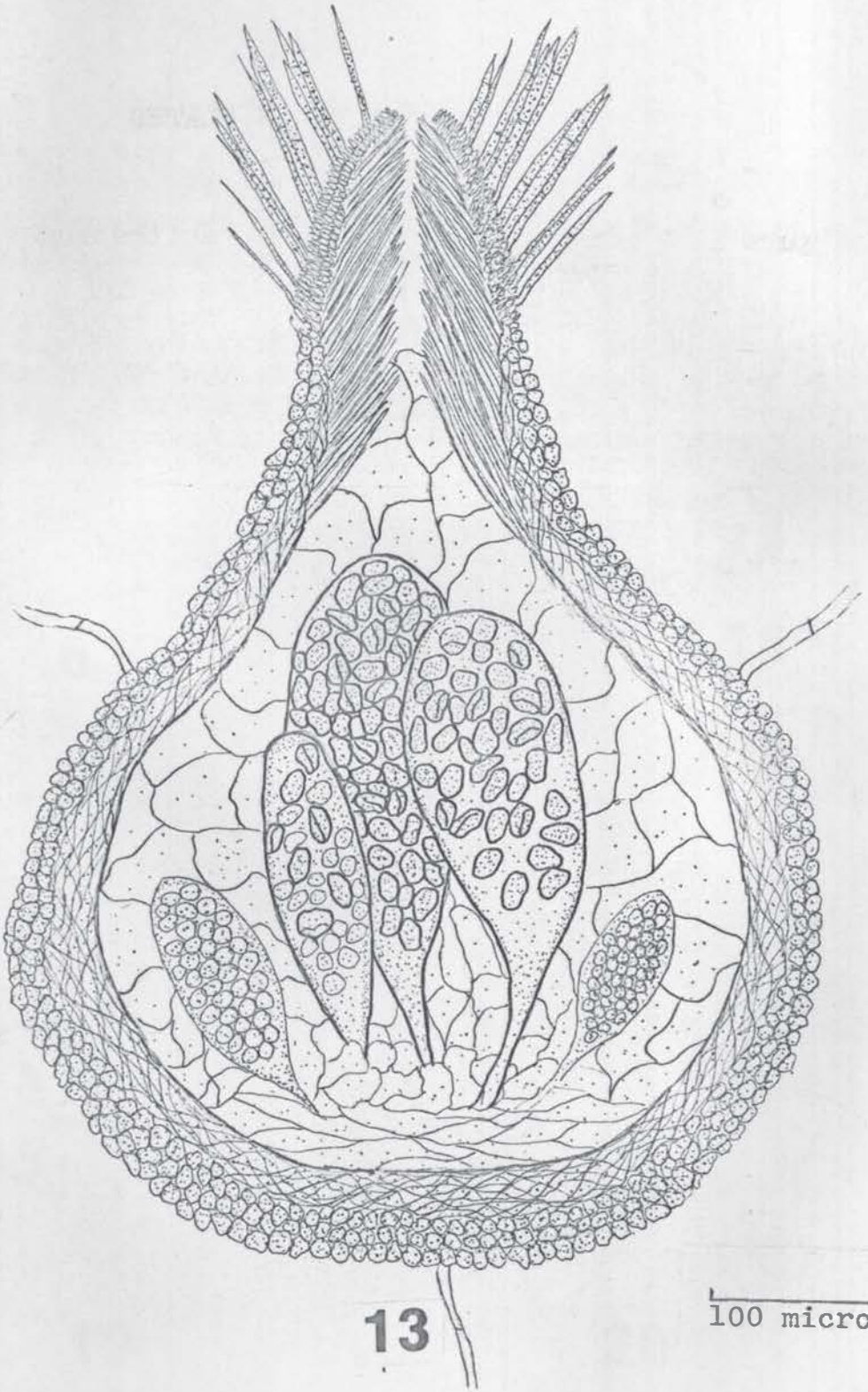


100 microns

12

EXPLANATION OF PLATES

Figure 13 Section of a mature perithecium showing developed asci, periphyses and setae. X125.



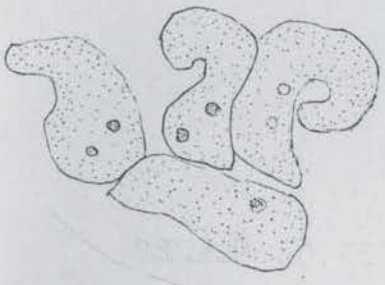
13

100 microns

EXPLANATION OF PLATES

Figures 14-15 — Figures showing the binucleate condition of ascogenous cells before crozier formation. X1250

Figures 16-20 — Croziers at different stages of development. Figure 20 indicating a young ascus and the fusion nucleus. X1250.



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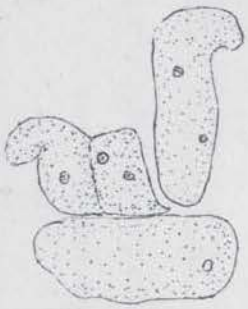

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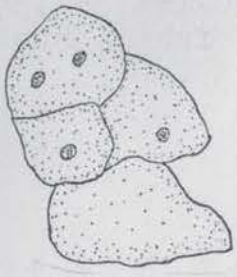

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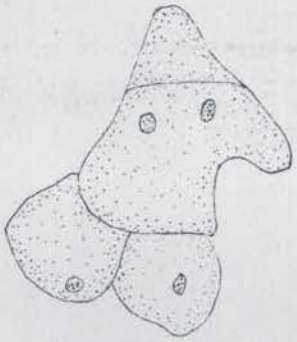

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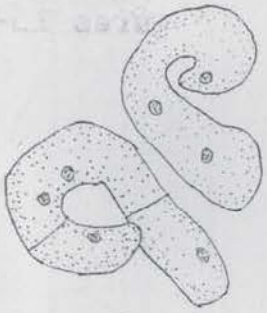

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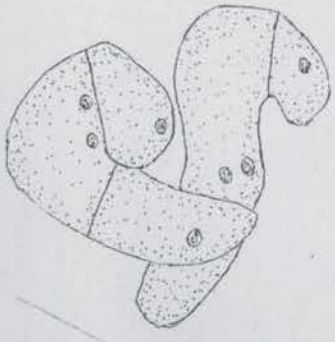

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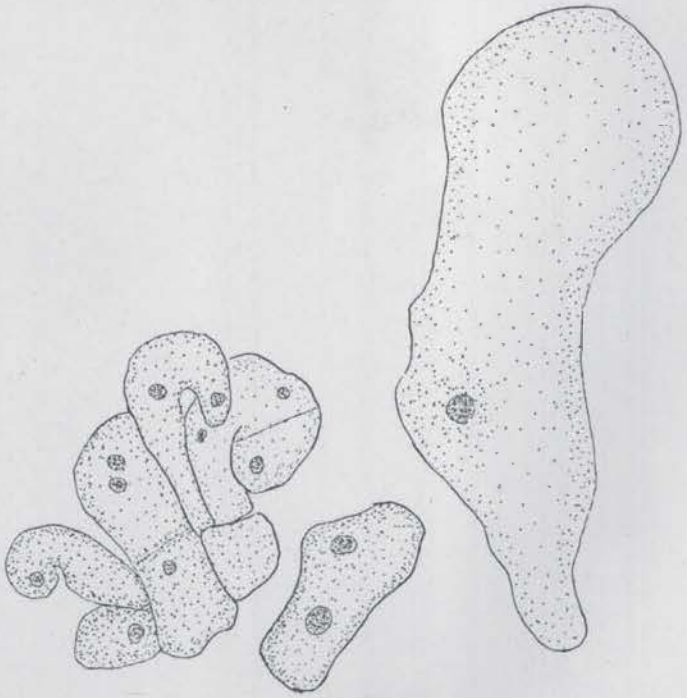

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EXPLANATION OF PLATES

Figure 21 Young ascogenous cells in the binucleate condition. X1000.

Figure 22 Mature ascospore with basal appendage attached. X1000.

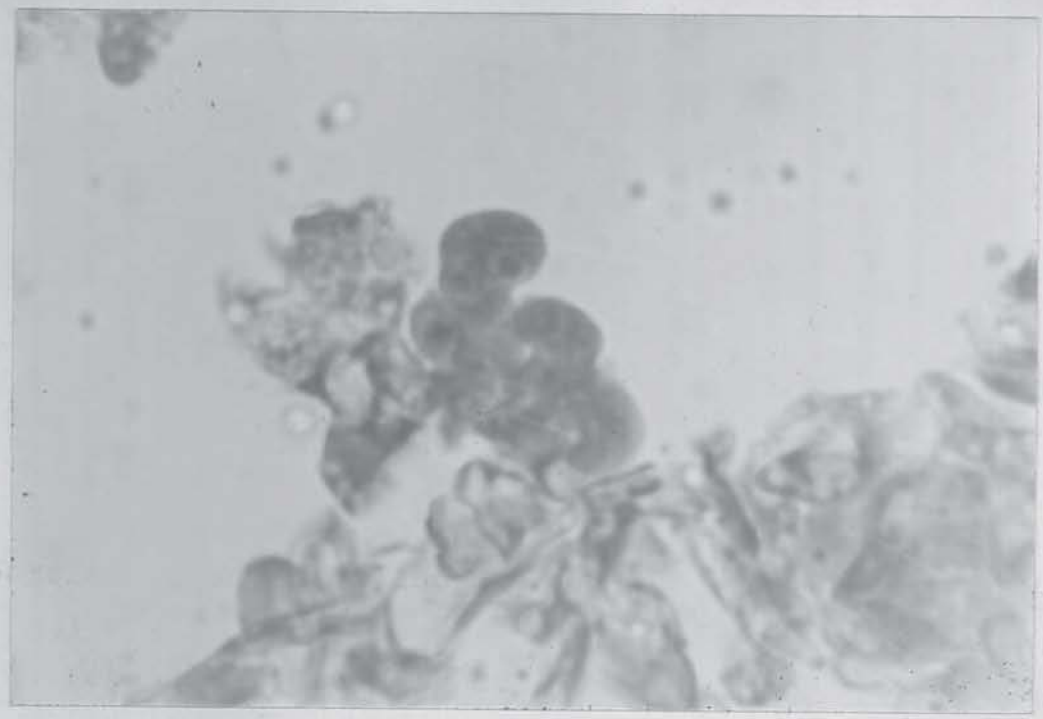

Fig. 21

Fig. 22

TABLE 1 DEHYDARTION SERIES USING TERTIARY BUTYL ALCOHOL(TBA) AND ETHYL ALCOHOL(ETOH)

Grade	Distilled Water	ETOH(95%)	TBA(100%)	Time in solution
1	95 mls	5 mls	-	2 hrs
2	90 mls	10 mls	-	2 hrs
3	80 mls	20 mls	-	2 hrs
4	70 mls	30 mls	-	2 hrs
5	50 mls	40 mls	10 mls	2 hrs
6	30 mls	50 mls	20 mls	2 hrs
7	15 mls	50 mls	35 mls	overnight
8	10 mls	40 mls	50 mls	1.5 hrs
9	-	25 mls	75 mls	1.5 hrs
10	-	-	100 mls*	overnight
11	-	-	100 mls	1.5 hrs
12	-	-	50 mls**	1.5 hrs

*Eosin dye was added.
**Paraffin oil (50 mls) was added.